REMARKS

Applicant respectfully requests reconsideration of this application in view of the amendments and remarks made herein.

Claims 1-37, 40-43, 45-46 and 48-49 are pending. Claims 22, 23 and 28-49 are canceled. Claims 12, 15-16, and 19-20 are withdrawn from consideration with the understanding that said claims will be reintroduced upon allowance of a generic claim. Claims 1, 2, 7, 9, 10, 21 and 24-27 have been amended to more particularly point out and distinctly claim the subject matter of the invention. Applicant respectfully submits that the amended claims are supported by the original disclosure of this application. As such, no new matter has been added by these amendments

1. Summary of the Invention

The present invention relates to a method for proteomic analysis of a heterogenous sample of proteins, or protein or peptide fragments. As disclosed in the specification, one of the disadvantages of prior art methods for proteomic analysis was the very large numbers of protein-specific binders required to achieve this analysis (see, page 3 lines 15-22). Specifically, the prior art methods used "binders" (i.e. molecules with a specific binding affinity) designed to be specific for individual proteins (or fragments derived therefrom) in a sample and so, for example, in order to analyze a sample of 2000 different proteins one needed to individually isolate each of those 2000 proteins in advance, generate a specific binder (e.g. an antibody) to each protein, and then produce an array with each of those specific binders immobilized thereon. Such prior art methods were time-consuming and labor-intensive and presupposed an advanced knowledge of the identity of individual proteins within a sample in order to generate a specific binder for each protein.

The present invention has overcome these disadvantages. For example, no advanced knowledge of the identity of individual proteins in a protein sample is required in order to

perform the method of the present invention because a standard array of binders can be used for any sample. Moreover, much lower numbers of different binders are required than the methods employed by the prior art. This is because the present invention employs binders that can each bind to peptides and protein and peptide fragments from divergent sources. Since the binders used in the present invention are not specific for an individual protein source, they can each bind multiple unrelated protein and peptide fragments. Accordingly, a less than 1:1 ratio between binders and proteins in a sample can be utilized. This makes the production and use of arrays much more economical and labor-efficient than those required to implement the prior art methods.

The present invention overcomes the disadvantages of the prior art methods by a unique interrelationship between the binding and characterizations steps. The binding steps of the present invention involve separating peptides, or protein or peptide fragments, into distinct classes irrespective of the parent proteins from which they were derived. The only distinguishing feature of each class is that the molecules bound in any given class will all contain the same motif, e.g. the same C-terminal tri- or tetra-peptide sequence. Thus, the variety of peptides, or protein of peptide fragments bound to a given type of binder can differ in sequence at all positions other than the common motif, i.e. a heterogeneous class of peptides, or protein or peptide fragments, will be bound to any given type of binding molecule. As a result of this, lower numbers of binders are required in order to capture a useful proportion of peptides, or protein or peptide fragments, in a sample and, moreover, no advanced knowledge of the identity of proteins in a sample is required – on the contrary, a standard array can be used to analyze any sample of proteins.

However, because the peptides, or protein or peptide fragments, bound by each type of binding molecule are heterogeneous, their separation during the binding step is incomplete. Such incomplete separation could not be tolerated by prior art methods (e.g., including the

prior art references Minden and Barry cited by the Examiner) because they rely on binding patterns created on an array to identify individual proteins.

However, in the case of the present invention, the incomplete separation during the binding step of peptides, and protein and peptide fragments, derived from different parent proteins (i.e. the creation of heterogeneous classes) can be tolerated because of the further characterization step. The molecules in each class are characterized to determine the different masses of molecules in each class and the relative abundance of molecules of each mass in a given class. Thus, molecules derived from different parent proteins are distinguished on the basis of mass and abundance.

By combining the information derived from the binding and characterization steps it is possible to derive a picture of the protein sample analyzed, through a simple and time-efficient method that requires no advanced knowledge of the individual proteins in the sample being analyzed and employs only a single binding affinity step.

2. Drawings

The Drawings are objected to by the Examiner as failing to comply with 37 CFR 1.84(p)(5) because Figures 2-14 are described together. In response, applicants have amended the specification to insert a section entitled "Brief Description of Figures" which sets forth separate figure legends corresponding to figures 1-14. Additionally, figure legends 2-14 specify the specific binding molecule used for capture of peptides. Support for the specified binding element can be found in Figures 2-14 as filed, which indicated in the left hand corner of the Figure, the binding molecule utilized for capture of peptides. Applicant asserts that, in view of the new figure legends, the objection of the Examiner should be withdrawn.

3. **The Rejections Under 35 U.S.C. § 112, Second Paragraph Should be Withdrawn**

Claims 1-11,13-14,17-18, and 21-27 are rejected under 35 U.S.C. §112, second paragraph, as being indefinite for allegedly failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

According to the Examiner, Claim 1 is vague and indefinite because a nexus between method step (a) and method step (b) is missing. The Examiner maintains that if the heterogenous sample of proteins, peptides, or fragments can be separated into classes via a common motif then it would appear that the proteins, peptides, or fragments have already been characterized to an extent via a common motif. In addition, the Examiner asserts that if the proteins, peptides, or fragments can be classified via binding members then it would appear that the proteins, peptides, or fragments have already been classified to an extent. Thus, claim 1 and all its dependent claims are rejected under 35 U.S.C. §112, second paragraph.

To further clarify Claim 1, step (b) has been amended to specify that a determination is made of the mass and abundance of peptides, or protein or peptide fragments, in the heterogeneous classes. Support for amended claim 1 can be found, for example, on page 23, lines 8-13, and in Claims 22 and 23 of the application as filed.

Applicant asserts that Claim 1, as amended, is clear and concise, therefore, the rejection under 35 U.S.C.§ 112, second paragraph, should be withdrawn.

4. **The Rejections Under 35 U.S.C.§ 102(e) Should Be Withdrawn**

Claims 1-11, 13-14, 17-18, 21-22, 25, and 27 are rejected under 35 U.S.C. §102(e) as being anticipated by Minden et al. WO 02/086081 ("Minden").

According to the Examiner, Minden teaches methods of identifying a protein via assigning (e.g. separating) binding reagents to designated locations on an array, detecting the binding patterns, and comparing the binding pattern to a reference set. Minden is alleged to

further teach (i) that the total protein content of a cell or tissue can be utilized as the protein mixture; (ii) that the protein mixture can be fragmented with various chemical or enzymatic methods including trypsin; (iii) that trypsin cleavage forms a peptide or epitope (e.g. motif) with C-terminal lysine or arginine residues; (iv) that the peptides or epitopes (e.g. motifs) can be at least three amino acids in length and can have at least two variable amino; (v) that arrays can have different binding molecules at spatially addressable locations which bind to different binding reagents; that the protein mixture may comprise all (e.g. 100%) of the proteins and that the epitopes cover the binding mixture; (vi) that the array can have 2-100 different proteins; (vii) that the binding reagents can be antibodies; (viii) that the proteins can be compared to a reference set; (ix) that the molecular weight or mass of the binding reagents can be determined and that spectrometry can be utilized; (x) that the reference set can include prediction about binding based on the predicted digests of a protein mixture (e.g. unfragmented); and (xi) that various binding reagents can be compared to a reference set or to other binding reagents. Therefore, according to the Examiner, the presently claimed invention is anticipated in view of the teachings of Minden.

In order for a reference to anticipate a claim, each and every element of the claim must be disclosed in that one reference. *Orthokinetics, Inc. v. Safety Travel Chairs, Inc.*, 806 F.2d 1565 (Fed. Cir. 1985). "Anticipation under Section 102 can be found only if a reference shows exactly what is claimed. . ." *Structural Rubber Prod. Co. v. Park Rubber Co.*, 749 F.2d 707 (Fed. Cir. 1984).

Claim 1 has been amended to specify that step (b) includes a determination of the mass and abundance of peptides, or protein or peptide fragments, in the heterogeneous classes. Given that Minden fails to disclose such a step, Minden cannot anticipated the

presently claimed invention.[1] Applicant respectfully requests that the rejection under 35 U.S.C.§ 102(e) be withdrawn.

5. The Rejections Under 35 U.S.C. §103 Should be Withdrawn

Claims 1-11,13-14,17-18, and 21-27 are rejected under 35 U.S.C. §103(a) as being unpatentable over Minden and Barry et al. WO 0225287 ("Barry").

According to the Examiner, Minden teaches methods of identifying a protein via assigning (e.g. separating) binding reagents to designated locations on an array, detecting the binding patterns, and comparing the binding pattern to a reference set (e.g. characterizing; please refer to the abstract, paragraphs. Minden is alleged to further teach (i) that the total protein content of a cell or tissue can be utilized as the protein mixture; (ii) that the protein mixture can be fragmented with various chemical or enzymatic methods including trypsin; (iii) that trypsin cleavage forms a peptide or epitope (e.g. motif) with C-terminal lysine or arginine residues; (iv) that the peptides or epitopes (e.g. motifs) can be at least three amino acids in length and can have at least two variable amino; (v) that arrays can have different binding molecules at spatially addressable locations which bind to different binding reagents; that the protein mixture may comprise all (e.g. 100%) of the proteins and that the epitopes cover the binding mixture; (vi) that the array can have 2-100 different proteins; (vii) that the binding reagents can be antibodies; (viii) that the proteins can be compared to a reference set; (ix) that the molecular weight or mass of the binding reagents can be determined and that spectrometry can be utilized; (x) that the reference set can include prediction about binding based on the predicted digests of a protein mixture (e.g. unfragmented); and (xi) that various binding reagents can be compared to a reference set or to other binding reagents.

The Examiner alleges that, although Minden does not specifically teach determining the abundance of the proteins or the use of desorption mass spectrometry or collision induced

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[1] We note that the Examiner acknowledges on page 10, lines 7-9, of the Office Action that Minden does not disclose the determination of the abundance of proteins.

dissociation mass spectrometry, Barry teaches methods of (i) determining the binding and mass of trypsin digested proteins (including antibodies) from a cell (including phage) or tissue sample immobilized on an array (please refer to the abstract, pages 2-6, 21-30, Figures 3-6 and 8-10, Examples 2-3); (ii) determining the abundance of proteins via MALDI-TOF; MALDI-TOF (matrix assisted laser desorption ionization-time of flight) mass spectrometry (e.g. combination of both desorption mass spectrometry and collision induced dissociation mass spectrometry or CID); and (iii) determining the abundance of the protein.

According to the Examiner, it would have been obvious to a person of ordinary skill in the art at the time the invention was made to modify the method of identifying proteins taught by Minden with the MALDI-TOF analysis taught by Barry. Additionally, the Examiner states that one having ordinary skill in the art would have been motivated to do this because Barry teaches that the use of mass spectrometry and MALDI-TOF provide semi-quantitative and quantitative results for protein microarrays. Moreover, the Examiner maintains that one of ordinary skill in the art would have had a reasonable expectation of success in the modification of the method of identifying proteins taught by Minden with the MALDI-TOF analysis taught by Barry because of the examples provided by Barry showing that trypsin digested antibody arrays can be quantitated via MALDI-TOF. Therefore, according to the Examiner, the modification of the method of identifying proteins taught by Minden with the MALDI-TOF analysis taught by Barry render the instant claims *prima facie* obvious.

Applicant submits that the Office has not set forth a *prima facia* case of obviousness. A finding of obviousness under 35 U.S.C. § 103 requires a determination of: (1) the scope and content of the prior art; (2) the level of ordinary skill in the art; (3) the difference between the claimed subject mater and the prior art; and (4) whether the differences are such that the subject matter as a whole would have been obvious to one of ordinary skill in the art at the time the invention was made. *Graham v. Deere* 383 U.S. 1 (1966). Further, the prior art

relied upon by an Examiner to establish a *prima facie* case must not only suggest that the claimed method be performed, but the prior art must also provide one of ordinary skill in the art with a reasonable expectation that the claimed subject matter can be successfully used to effect a practical purpose. *In re Vaeck*, 20 USPQ2d 1438, 1442 (Fed. Cir. 1991).

Applicants assert that the issue of obviousness must be determined in light of the scope and content of the prior art. As explained in detail below both of Minden and Barry teach away from the presently claimed invention. Specifically, both Minden and Barry teach a method that requires advanced knowledge of the identity of proteins in a sample, such that specific binders for each protein (or fragment thereof) can be separated, thereby to produce homogeneous classes of proteins (or fragments thereof) as bound to an array. This is in direct contrast to the present invention which requires the generation of a heterogeneous class of peptides or protein or peptide fragments bound to the different types of binding molecules on an array.

The method of the present invention provides for the analysis of large numbers of proteins in a sample, such that the ratio of binders to proteins analyzed can be less than 1:1. For example, see page 3, lines 19-22 of the specification, which describes the use of only 200 binders to analyze 10,000 different peptides (i.e. a ratio of 0.02:1). Thus, a rapid and relatively simple method for the quantification of the proteins of an unknown complex sample is provided by the present invention. The low number of binders means that there are fewer spots on the array to characterize (e.g. several proteins can be characterized by mass spectrometry analysis of a single spot), and so the characterization step is substantially simplified in comparison to a method where the characterization of each protein can require analysis of at least one spot (and possibly several spots) on the array. This approach is possible because of the unique inter-relationship between the binding and characterization steps employed in the method of the present invention which tolerates the production of heterogeneous classes of peptides, or protein or peptide fragments, as bound to the array.

The reduction in the number of binders is made possible because the heterogeneous classes of peptides bound to the binders are further characterized to determine the mass and abundance of individual members of the class (e.g. by mass spectrometry). Thus, the user requires no advanced knowledge of the individual proteins in a sample prior to its analysis, nor does an array have to be specifically adapted to allow for the analysis of particular protein samples — rather a standard array can be used to analyze any protein sample.

In contrast, Minden refers to a method for identification of a protein, in a protein mixture or protein catalogue, that relies on having advanced knowledge of a protein of interest in order to design suitable binders with which to analyze that protein. For example, Minden, at page 13, paragraph [0035], defines a "protein mixture" for analysis as "a composition of proteins wherein the identity of all, or substantially all, of the proteins in the composition are known."

Applicant asserts that Minden requires the user to know the identity of all (or substantially all) proteins within a sample to be analyzed. In contrast, the method of the present invention does not suffer from this disadvantage as the utilized binding agents are not specific for any particular protein type and, therefore, can be used generally to analyze any protein sample without advanced knowledge of the individual proteins within a given sample.

This difference is further highlighted by Minden's mode of analysis of his results. For example, the final step of Claim 1 of Minden, reads as follows:

> "...comparing the binding pattern of the peptide fragments to a reference set".

Thus, in order to utilize the method of Minden, it is necessary to know in advance which peptides are of interest (the "reference set") and determine what binding pattern would be expected from them under the same conditions. The method of the present invention requires no such advance knowledge and can be utilized to analyze samples with an unknown protein composition.

Furthermore, the method of Minden only allows the reader to analyze a small number of proteins at any one time. For example, on page 18, paragraph [0041], Minden states the following:

> "...and preferably permitting the deconvolution of a mixture of from 2 to about 10 or more proteins, typically from about 2 to 5 about proteins."

and on page 22, paragraph [0047] it is further stated:

> "...it may be possible to identify the proteins in a mixture containing from about 2 to about 10 or more different proteins, more typically from about 2 to 5 about different proteins, in a single assay it may be possible to differentiate even larger numbers of proteins... of from 2 to about 100 or more different proteins in a single assay"

Despite the small number of proteins to be analyzed by Minden, a relatively high number of binders are required. For example, on page 18, paragraph [0042] of Minden, it is stated that:

> "This set optimally includes (20 x 20) + (20 x 20) = 800 binding agents .

In other words, Minden teaches a method wherein up to 800 different specific binding agents are needed to analyze only 2 to 100 different proteins. This means that the ratio of binders to proteins analyzed using Minden's method can vary from 8:1 to 400:1. This is in comparison to the ratio of 0.02:1 as exemplified by the present application. Thus, the method of the present invention represents a significant advancement over the teaching of Minden, in that it allows the production and analysis of arrays for proteomic analysis to be markedly simplified.

In summary, by following the teaching of Minden, the person skilled in the art is unable to take advantage of the analysis of heterogeneous classes of bound peptides, or protein or peptide fragments, in order to simplify array production and analysis. The reason for this is clear from the mention in Minden of the need to compare "binding patterns" with those of a reference set (as set forth in Claim 1). The fact that a binding "pattern" is required means that each protein analyzed by the method of Minden must contribute multiple bound fragments (since individual bound fragments cannot produce a "pattern"). In other words, the

method of Minden needs to include multiple binders for each whole protein. There is no such requirement for practice of the present invention (for example, see page 6, lines 22-25). There is no indication in Minden that the analysis can proceed without determining the binding pattern created by a sample. Thus, a person of skill in the art following Minden's teaching could not reduce the number of binders required.

Additionally, the method of the present invention provides information on the abundance of proteins. Information on the abundance of proteins (i.e. quantitative information) in a sample can be just as, if not more, important than information on the presence or absence of a particular protein (i.e. qualitative information). For example, a disease-related protein may be expressed in both a disease and non-disease state, but at different levels.

Applicant asserts that there is nothing in Minden to suggest that quantification of proteins in the sample is desirable, much less possible. The method of Minden would only provide qualitative data and so would not identify the disease-related protein as being related to the disease in a comparison between diseased and non-diseased samples. By contrast, the quantitative information provided by the method of the present invention would clearly show that the expression levels of the protein differed between disease and non-disease state and so would identify the protein as a disease-related protein.

The Examiner has referred to Barry as motivation to adapt the teaching of Minden to include a step of determining abundance. However, this is irrelevant to the obviousness of the present invention because, as explained above, Minden teaches away from the present invention, and towards the production of homogeneous, not heterogeneous, protein classes as bound to an array, using an array wherein more than one binding agent is required per protein to be characterized. Barry has not been cited to supplement this deficiency in the teaching of Minden, nor does it do so.

The Examiner has not provided any indication of why a person skilled in the art would consult Barry as a means of adapting Minden. In the absence of an explicit teaching or suggestion in Minden that determining the abundance of proteins could or should be done, the skilled reader of Minden would not be motivated to search for, or consider Barry. The Examiner appears to have selected those documents with the benefit of hindsight knowledge of the invention, which is not permissible.

Even if, for the sake of argument, the skilled person did consider Minden and Barry in combination, he would not be motivated to develop a method as defined by Claim 1 of the present application. On the contrary, the person skilled in the art would adapt the method of Minden to be more like that of Barry and, in doing so, would arrive at a method that is even more different to the claimed method.

Specifically, Barry provides the reader with a method of proteomic analysis that relates to a method in which each binder corresponds to one protein and requires advanced knowledge of proteins in the sample in order to generate an appropriate array of binders. For example, see Barry, page 4, lines 2-4:

> "Each capture agent specifically recognizes a corresponding peptide compound and its corresponding target peptide fragment from a different protein of interest" (emphasis added)"

page 3, lines 22-24:

> "Fragmentation of such complex structures results in a plurality of smaller units (peptides) which are nevertheless representative of and unique to each fragmented protein. By selecting target peptide fragments which are unique to each protein of interest and further selecting... it is possible...." (emphasis added).

page 2, lines 32-37:

> "The array may consist of capture agents selected to bind one or more peptide fragments derived from each protein of interest. Preferably, a plurality of target peptide fragment/capture agent combinations is employed for each protein, thus increasing the confidence of an analytical signal response".

and page 12, lines 22-23:

> "Once proteins of known sequence are identified, it is then possible to design peptide compounds, prepare capture agents, prepare arrays comprising capture agents....." (emphasis added).

Thus, Barry teaches one binder for one protein, or even several binders for one protein. Thus, the peptide fragments are unique to each fragmented protein. As a result, each class bound on the array is homogeneous, rather than heterogeneous (the latter of which is specified in Claim 1 of the present application).

As with Minden, the method of Barry requires an advanced knowledge of each protein in the sample in order to prepare the necessary binders and also requires a large number of different binders to be used. This is in contrast with the present invention which provides a method that is capable of capturing, from an unknown complex mixture, peptides corresponding to several proteins with one binder, i.e. using a low number of binders.

Thus, based on the disclosure of Minden, in combination with the teachings of Barry, one skilled in the art would be motivated to adapt Minden's method in such a way as to use specific binders on an array, thereby producing homogeneous classes of proteins or peptides bound to an array, and thereby retaining (or even increasing) the number of specific binders required on an array for use in Minden's method. Accordingly, the combined teaching of Minden and Barry would motivate the person skilled in the art to utilize a method that is technically different, and has considerable disadvantages, compared to the method of Claim 1 of the present invention.

Finally, there is no indication in Barry that it is even desirable, much less possible, to characterize a protein by using an array that comprises binders that bind different peptides corresponding to several different proteins at each spot. On the contrary, the skilled person upon reading Barry would continue to produce arrays that result in the production of homogeneous classes of bound proteins. This is in direct contrast to the method of Claim 1 of the present application, which specifies the production of heterogeneous classes.

Applicants assert that a *prima facia* case of obviousness has not been established. In light of these remarks, Applicant respectfully requests that the obviousness rejections be withdrawn.

CONCLUSION

In view of the foregoing amendments and remarks, it is believed that the subject claims are in condition for allowance, which action is earnestly solicited. If, in the opinion of the Examiner, a telephone conference would expedite prosecution of the subject application, the Examiner is invited to call the undersigned attorney.

Respectfully submitted,

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